

## Claims

### What is claimed is:

5 1. A method for producing a heterologous polypeptide, comprising:

(a) cultivating a mutant of a parent filamentous fungal cell under conditions conducive for the production of the heterologous polypeptide, wherein (i) the mutant comprises a first nucleic acid sequence encoding the heterologous polypeptide, and (ii) the mutant produces less of a cyclohexadepsipeptide than the parent filamentous fungal cell when  
10 cultured under the same conditions; and

(b) isolating the heterologous polypeptide from the cultivation medium.

15 2. The method of claim 1, wherein the filamentous fungal cell is an *Acremonium*, *Aspergillus*, *Aureobasidium*, *Cryptococcus*, *Filibasidium*, *Fusarium*, *Gibberella*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Myrothecium*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Piromyces*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, or *Trichoderma* cell.

20 3. The method of claim 1, wherein the filamentous fungal cell is a *Fusarium* cell.

4. The method of claim 3, wherein the *Fusarium* cell is a *Fusarium venenatum* cell.

25 5. The method of claim 4, wherein the *Fusarium venenatum* cell is *Fusarium venenatum* ATCC 20334.

6. The method of claim 4, wherein the *Fusarium venenatum* cell is a morphological mutant.

30 7. The method of claim 6, wherein the *Fusarium venenatum* cell is a morphological mutant of *Fusarium venenatum* ATCC 20334.

a

claim 1  
8. The method of ~~any of claims 1-7~~, wherein the mutant cell comprises a second nucleic acid sequence which comprises a modification of at least one of the genes involved in the production of the cyclohexadepsipeptide.

5 9. The method of claim 8, wherein the genes are selected from the group consisting of a cyclohexadepsipeptide synthetase gene, enniatin synthetase gene, and D-hydroxyisovalerate dehydrogenase gene.

10 10. The method of claim 8, wherein one of the genes is a cyclohexadepsipeptide synthetase gene.

11. The method of claim 8, wherein one of the genes is an enniatin synthetase gene.

12. The method of claim 8, wherein one of the genes is a D-hydroxyisovalerate dehydrogenase gene.

claim 1  
13. The method of ~~any of claims 1-12~~, wherein the mutant cell produces at least about 25% less of the cyclohexadepsipeptide than the parent filamentous fungal cell when cultured under identical conditions.

14. The method of any of claims 1-13, wherein the mutant cell produces no cyclohexadepsipeptide.

25 15. The method of any of claims 1-14, wherein the filamentous fungal cell comprises at least two copies of the first nucleic acid sequence.

16. The method of any of claims 1-15, wherein the heterologous polypeptide is a hormone, hormone variant, enzyme, ~~receptor~~ or portion thereof, antibody or portion thereof, or reporter.

30 17. The method of claim 16, wherein the enzyme is an oxidoreductase, transferase,

hydrolase, lyase, isomerase, or ligase.

18. The method of claim 17, wherein the enzyme is an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, or xylanase.

19. The method of any of claims 1-18, wherein the mutant cell further comprises one or more modifications of one or more third nucleic acid sequences, wherein the modification reduces or eliminates expression of the one or more third nucleic acid sequences.

20. The method of claim 19, wherein the third nucleic acid sequence encodes an enzyme selected from the group consisting of an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, and xylanase.

21. The method of any of claims 19, wherein the third nucleic acid sequence encodes a protease.

22. A cyclohexadepsipeptide-deficient mutant of a filamentous fungal cell, comprising a first nucleic acid sequence encoding a heterologous polypeptide, wherein the mutant produces less of a cyclohexadepsipeptide than the parent filamentous fungal cell of the mutant cell when cultured under the same conditions.

23. The mutant cell of claim 22, wherein the mutant cell comprises a second nucleic acid sequence which comprises a modification of at least one of the genes involved in the

production of the cyclohexadepsipeptide.

24. The mutant cell of claim 23, wherein the genes are selected from the group consisting of a cyclohexadepsipeptide synthetase gene, enniatin synthetase gene, and D-hydroxyisovalerate dehydrogenase gene.

25. The mutant cell of claim 23, wherein one of the genes is a cyclohexadepsipeptide synthetase gene.

26. The mutant cell of claim 23, wherein one of the genes is an enniatin synthetase gene.

27. The mutant cell of claim 23, wherein one of the genes is a D-hydroxyisovalerate dehydrogenase gene.

28. The mutant cell of any of claims 22-27, wherein the cell comprises at least two copies of the first nucleic acid sequence.

29. A method for obtaining the mutant cell of any of claims 22-28, comprising:

(a) introducing into a parent filamentous fungal cell a first nucleic acid sequence encoding a heterologous polypeptide and a second nucleic acid sequence comprising a modification of at least one of the genes responsible for the production of a cyclohexadepsipeptide; and

(b) identifying the mutant from step (a), wherein the mutant produces less of the cyclohexadepsipeptide than the parent filamentous fungal cell of the mutant cell when cultured under the same conditions.

30. An isolated cyclohexadepsipeptide synthetase, selected from the group consisting of:

(a) a cyclohexadepsipeptide synthetase having an amino acid sequence which has at least 65% identity with the mature polypeptide contained within SEQ ID NO:2;

(b) a cyclohexadepsipeptide synthetase which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) the nucleic acid

sequence of SEQ ID NO:1, (ii) the cDNA sequence of SEQ ID NO:1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii);

(c) an allelic variant of (a) or (b); and

(d) a fragment of (a), (b), or (c) that has cyclohexadepsipeptide synthetase activity.

31. The cyclohexadepsipeptide synthetase of claim 30, having an amino acid sequence which has at least 65% identity with the mature polypeptide contained within SEQ ID NO:2.

32. The cyclohexadepsipeptide synthetase of claim 31, having an amino acid sequence which has at least 70% identity with the mature polypeptide contained within SEQ ID NO:2.

33. The cyclohexadepsipeptide synthetase of claim 32, having an amino acid sequence which has at least 80% identity with the mature polypeptide contained within SEQ ID NO:2.

34. The cyclohexadepsipeptide synthetase of claim 33, having an amino acid sequence which has at least 90% identity with the mature polypeptide contained within SEQ ID NO:2.

35. The cyclohexadepsipeptide synthetase of claim 34, having an amino acid sequence which has at least 95% identity with the mature polypeptide contained within SEQ ID NO:2.

36. The cyclohexadepsipeptide synthetase of claim 30, comprising the amino acid sequence of SEQ ID NO:2.

37. The cyclohexadepsipeptide synthetase of claim 30, consisting of the amino acid sequence of SEQ ID NO:2 or a fragment thereof.

38. The cyclohexadepsipeptide synthetase of claim 37, consisting of the amino acid sequence of SEQ ID NO:2.

39. The cyclohexadepsipeptide synthetase of claim 38, which consists of the mature

polypeptide contained within SEQ ID NO:2.

40. The cyclohexadepsipeptide synthetase of any of claims 30-39, which is obtained from a *Fusarium* strain.

41. The cyclohexadepsipeptide synthetase of claim 40, which is obtained from *Fusarium venenatum* strain.

42. The cyclohexadepsipeptide synthetase of claim 30, which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, (ii) the cDNA sequence of SEQ ID NO:1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii);.

43. The cyclohexadepsipeptide synthetase of claim 42, which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, (ii) the cDNA sequence of SEQ ID NO:1, or (iii) a complementary strand of (i) or (ii).

44. The cyclohexadepsipeptide synthetase of claim 42 or 43, which is obtained from a *Fusarium* strain.

45. The cyclohexadepsipeptide synthetase of claim 44, which is obtained from *Fusarium venenatum* strain.

46. The cyclohexadepsipeptide synthetase of claim 30, which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, (ii) the cDNA sequence of SEQ ID NO:1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii).

47. The cyclohexadepsipeptide synthetase of claim 46, which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with (i) the nucleic acid

sequence of SEQ ID NO:1, (ii) the cDNA sequence of SEQ ID NO:1, or (iii) a complementary strand of (i), (ii), or (iii);.

48. The cyclohexadepsipeptide synthetase of claim 46 or 47, which is obtained from a *Fusarium* strain.

49. The cyclohexadepsipeptide synthetase of claim 48, which is obtained from *Fusarium venenatum* strain.

50. The cyclohexadepsipeptide synthetase of claim 30, which is encoded by the nucleic acid sequences contained in plasmid pZL-ESA, which is contained in *Escherichia coli* NRRL B-30068, plasmid pZL-ESB, which is contained in *Escherichia coli* NRRL B-30069, and plasmid pZL-ESC, which is contained in *Escherichia coli* NRRL B-30070.

51. The cyclohexadepsipeptide synthetase of any of claims 30-50 which has at least 20% of the enzyme activity of SEQ ID NO:2.

52. A cyclohexadepsipeptide synthetase having the same enzyme activity as the cyclohexadepsipeptide synthetase of any of claims 30-51.

53. An isolated nucleic acid sequence comprising a nucleic acid sequence which encodes the cyclohexadepsipeptide synthetase of ~~any of claims 30-52~~ <sup>claim 30</sup>.

54. An isolated nucleic acid sequence comprising a nucleic acid sequence having at least one mutation in the mature cyclohexadepsipeptide synthetase coding sequence of SEQ ID NO:1, in which the mutant nucleic acid sequence encodes a cyclohexadepsipeptide synthetase consisting of the mature polypeptide contained within SEQ ID NO:2.

55. An isolated nucleic acid sequence produced by (a) hybridizing a DNA under medium stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, (ii) the cDNA sequence of SEQ ID NO:1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv)

a complementary strand of (i), (ii), or (iii); and (b) isolating the nucleic acid sequence.

56. The isolated nucleic acid sequence of claim 55 produced by (a) hybridizing a DNA under high stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, (ii) the  
5 cDNA sequence of SEQ ID NO:1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii), or (iii); and (b) isolating the nucleic acid sequence.

57. A nucleic acid construct comprising the nucleic acid sequence of claim 53 operably linked to one or more control sequences that direct the production of the  
10 cyclohexadepsipeptide synthetase in a suitable expression host.

58. A recombinant expression vector comprising the nucleic acid construct of claim 57.

59. A recombinant host cell comprising the nucleic acid construct of claim 57.

60. A method for producing a mutant nucleic acid sequence, comprising (a) introducing at least one mutation into the mature cyclohexadepsipeptide synthetase coding sequence of SEQ ID NO:1, wherein the mutant nucleic acid sequence encodes a cyclohexadepsipeptide synthetase consisting of the mature polypeptide contained within SEQ ID NO:2; and (b)  
20 recovering the mutant nucleic acid sequence.

61. A mutant nucleic acid sequence produced by the method of claim 60.

62. A method for producing a cyclohexadepsipeptide synthetase, comprising (a)  
25 cultivating a strain comprising the mutant nucleic acid sequence of claim 61 encoding the cyclohexadepsipeptide synthetase to produce a supernatant comprising the cyclohexadepsipeptide synthetase; and (b) recovering the cyclohexadepsipeptide synthetase.

63. A method for producing the cyclohexadepsipeptide synthetase of <sup>claim 30</sup> ~~any of claims 30-52~~  
30 comprising (a) cultivating a strain to produce a supernatant comprising the cyclohexadepsipeptide synthetase; and (b) recovering the cyclohexadepsipeptide synthetase.



claim 30

64. A method for producing the cyclohexadepsipeptide synthetase of ~~any of claims 30-52~~ comprising (a) cultivating a host cell comprising a nucleic acid construct comprising a nucleic acid sequence encoding the cyclohexadepsipeptide synthetase under conditions suitable for production of the cyclohexadepsipeptide synthetase; and (b) recovering the cyclohexadepsipeptide synthetase.

65. A method for producing a cyclohexadepsipeptide synthetase comprising (a) cultivating a host cell under conditions conducive for production of the cyclohexadepsipeptide synthetase, wherein the host cell comprises a mutant nucleic acid sequence having at least one mutation in the mature cyclohexadepsipeptide synthetase coding sequence of SEQ ID NO:1, wherein the mutant nucleic acid sequence encodes a cyclohexadepsipeptide synthetase consisting of the mature polypeptide contained within SEQ ID NO:2, and (b) recovering the cyclohexadepsipeptide synthetase.

66. A method for producing a cyclohexadepsipeptide, comprising:

- (a) reacting the cyclohexadepsipeptide synthetase of any of claims 30-52 with D-2-hydroxyisovaleric acid, a branched chain L-amino acid, S-adenosylmethionine, and ATP; and
- (b) isolating the cyclohexadepsipeptide from the reaction.

67. A cyclohexadepsipeptide produced by the method of claim 66.

68. A method for producing a cyclohexadepsipeptide, comprising:

- (a) cultivating a cell under conditions suitable for the production of the cyclohexadepsipeptide, wherein the cell comprises a nucleic acid sequence encoding (i) a cyclohexadepsipeptide synthetase having an amino acid sequence which has at least 65% identity with the mature polypeptide contained within SEQ ID NO:2; (ii) a cyclohexadepsipeptide synthetase which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with the nucleic acid sequence of SEQ ID NO:1 or its complementary strand, or a subsequence of SEQ ID NO:1 of at least 100

nucleotides; (iii) an allelic variant of (a) or (b); or (iv) a fragment of (a), (b), or (c) that has cyclohexadepsipeptide synthetase activity; and

(b) isolating the cyclohexadepsipeptide from the reaction.

5 69. A cyclohexadepsipeptide produced by the method of claim 68.